

PYRAZOLOPYRIDINES

AND METHODS OF MAKING AND USING THE SAME

This non-provisional application is a continuation application of PCT/US2003/027722, filed on September 5, 2003, which is a continuation-in-part and claims benefit of priority of U.S. Provisional application 60/408,811, filed September 6, 2002. The entire disclosure of each of the aforementioned patent applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

TGF β (Transforming Growth Factor β) is a member of a large family of dimeric polypeptide growth factors that includes activins, inhibins, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs) and mullerian inhibiting substance (MIS). TGF β exists in three isoforms (TGF β 1, TGF β 2, and TGF β 3) and is present in most cells, along with its receptors. Each isoform is expressed in both a tissue-specific and developmentally regulated fashion. Each TGF β isoform is synthesized as a precursor protein that is cleaved intracellularly into a C-terminal region (latency associated peptide (LAP)) and an N-terminal region known as mature or active TGF β . LAP is typically non-covalently associated with mature TGF β prior to secretion from the cell. The LAP- TGF β complex cannot bind to the TGF β receptors and is not biologically active. TGF β is generally released (and activated) from the complex by a variety of mechanisms including interaction with thrombospondin-1 or plasmin.

Following activation, TGF β binds at high affinity to the type II receptor (TGF β RII), a constitutively active serine/threonine kinase. The ligand-bound type II receptor phosphorylates the TGF β type I receptor (Alk 5) in a glycine/serine rich domain, which allows the type I receptor to recruit and phosphorylate downstream signaling molecules, Smad2 or Smad3. See, e.g., Huse, M. et al., *Mol. Cell.* 8: 671-682 (2001). Phosphorylated Smad2 or Smad3 can then complex with Smad4, and the entire hetero-Smad complex translocates to the nucleus and regulates transcription of various TGF β -responsive genes. See, e.g., Massagué, J. *Ann. Rev. Biochem. Med.* 67: 773 (1998).

Activins are also members of the TGF β superfamily which are distinct from TGF β in that they are homo- or heterodimers of activin β a or β b. Activins signal in a similar manner to

TGF β , that is, by binding to a constitutive serine-threonine receptor kinase, activin type II receptor (ActRIIB), and activating a type I serine-threonine receptor, Alk 4, to phosphorylate Smad2 or Smad3. The consequent formation of a hetero-Smad complex with Smad4 also results in the activin-induced regulation of gene transcription.

5 Indeed, TGF β and related factors such as activin regulate a large array of cellular processes, e.g., cell cycle arrest in epithelial and hematopoietic cells, control of mesenchymal cell proliferation and differentiation, inflammatory cell recruitment, immunosuppression, wound healing, and extracellular matrix production. See, e.g., Massagué, J. *Ann. Rev. Cell. Biol.* 6: 594-641 (1990); Roberts, A. B. and Sporn M. B. *Peptide Growth Factors and Their Receptors*,
10 95: 419-472 Berlin: Springer-Verlag (1990); Roberts, A. B. and Sporn M. B. *Growth Factors* 8:1-9 (1993); and Alexandrow, M. G., Moses, H. L. *Cancer Res.* 55: 1452-1457 (1995). Hyperactivity of TGF β signaling pathway underlies many human disorders (e.g., excess deposition of extracellular matrix, an abnormally high level of inflammatory responses, fibrotic disorders, and progressive cancers). Similarly, activin signaling and overexpression of activin is
15 linked to pathological disorders that involve extracellular matrix accumulation and fibrosis (see, e.g., Matsuse, T. et al., *Am. J. Respir. Cell Mol. Biol.* 13: 17-24 (1995); Inoue, S. et al., *Biochem. Biophys. Res. Comm.* 205: 441-448 (1994); Matsuse, T. et al, *Am. J. Pathol.* 148: 707-713 (1996); De Bleser et al., *Hepatology* 26: 905-912 (1997); Pawlowski, J.E., et al., *J. Clin. Invest.* 100: 639-648 (1997); Sugiyama, M. et al., *Gastroenterology* 114: 550-558 (1998); Munz,
20 B. et al., *EMBO J.* 18: 5205-5215 (1999)) and inflammatory responses (see, e.g., Rosendahl, A. et al., *Am. J. Respir. Cell Mol. Biol.* 25: 60-68 (2001)). Studies have shown that TGF β and activin can act synergistically to induce extracellular matrix (see, e.g., Sugiyama, M. et al., *Gastroenterology* 114: 550-558, (1998)). It is therefore desirable to develop modulators (e.g., antagonists) to signaling pathway components of the TGF β family to prevent/treat disorders
25 related to the malfunctioning of this signaling pathway.